INFECTION AND IMMUNITY, May 1984, p. 445–451
0019-9567/84/050445-07$02.00/0

Vol. 44, No. 2

Enhancement of Host Resistance Against *Listeria* Infection by *Lactobacillus casei*: Role of Macrophages

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Received 14 October 1983/Accepted 14 February 1984

Among the 10 species of the genus *Lactobacillus*, *L. casei* showed the strongest protective action against *Listeria monocytogenes* infection in mice. The activity of *L. casei* differed with regard to the dose of administration. The anti-*L. monocytogenes* resistance in mice intravenously administered 5.5×10^7, 2.8×10^8, or 1.1×10^9 *L. casei* cells was most manifest at ca. 2, 2 and 13, and 3 to 21 days after its administration, respectively. The growth of *L. monocytogenes* in the liver of mice injected with *L. casei* (10^7, 10^8, or 10^9 cells) 48 h after infection was suppressed, particularly when 10^8 or 10^9 *L. casei* cells were given 2 or 13 days before the induced infection, respectively. This suppression of *L. monocytogenes* growth was overcome by carrageenan treatment or X-ray irradiation. [^{3}H]thymidine incorporation into the liver DNA increased 13 days after administration of *L. casei*, and augmentation of [^{3}H]thymidine incorporation during 6 to 48 h after infection was dependent on the dose of *L. casei*. Peritoneal macrophage accumulation observed 1 to 5 days after intraperitoneal injection of UV-killed *L. monocytogenes* was markedly enhanced when the mice were treated with *L. casei* cells 13 days before macrophage elicitation. Therefore, the enhanced host resistance by *L. casei* to *L. monocytogenes* infection may be mediated by macrophages migrating from the blood stream to the reticuloendothelial system in response to *L. casei* injection before or after *L. monocytogenes* infection.

Microorganisms such as mycobacteria (9, 23), corynebacteria (9, 17, 30), streptococci (15, 25), lactobacilli (11, 26, 27), and their components (7, 24) are known to be potent immunostimulants in malignancies (11, 15, 17) and in various infections (7, 9, 23, 25–27, 30). These organisms directly activate macrophage functions (11, 24, 32) and also enhance T or B cell-mediated macrophage activation (2, 14, 23). Studies on the enhancement of the anti-*Listeria monocytogenes* host resistance by *Corynebacterium parvum* (32) revealed the importance of the nonspecific activation of macrophages by this agent in overcoming the early growth of pathogens by host-defense mechanisms.

Bloksma et al. (3) reported that some lactobacilli, especially *Lactobacillus plantarum*, have a strong adjuvanticity in both delayed-type hypersensitivity (DTH) and antibody production to sheep erythrocytes, and this is mediated by a certain function of the reticuloendothelial system. Kato et al. (11) reported that *Lactobacillus casei* may induce macrophages which possess antitumor activity against sarcoma-180 and L 1210 leukemia. Previous studies in our laboratory showed that *L. casei* enhanced the resistance of mice to experimental infections due to extracellular parasites such as *Pseudomonas aeruginosa*, *Escherichia coli*, and others (26, 27). This enhancement of host resistance to various infections may ultimately depend on the generation of activated macrophages by either T cell-mediated immunological processes or by direct interactions with certain immunopotentiators (4, 6, 14, 21, 23, 31).

In the present study, I examined in mice the protective effects of lactobacilli, particularly *L. casei*, on experimental infection due to *L. monocytogenes*, a facultative intracellular parasite.

MATERIALS AND METHODS

Animals. Female 5-week-old ddY mice, purchased from the Shizuoka Union for Experimental Animals, Shizuoka, Japan, were used.

Bacteria. *Lactobacillus* spp. used were as follows: *L. lactis* ATCC 12315, *L. bulgaricus* ATCC 11842, *L. acidophilus* ATCC 19992, *L. salivarius* ATCC 11742, *L. plantarum* ATCC 8014, *L. fermentum* ATCC 9338, *L. cellobiosus* ATCC 11739, *L. brevis* ATCC 14869, *L. buchneri* ATCC 4005, and *L. casei* strains ATCC 393, ATCC 7469, YIT 0003, YIT 0006, YIT 0091, YIT 0112, and YIT 9018. These bacteria were obtained from S. Kodaira, Yakult Central Institute for Microbiological Research, Tokyo, Japan. Unless otherwise noted, viable *L. casei* YIT 0003 was used throughout the experiments. Organisms grown in Rogosa liquid medium (5) at 37°C for 18 h were washed twice with phosphate-buffered saline (PBS) (pH 7.2) and suspended in PBS, and the optical density was adjusted to the desired values at 540 nm on Spectronic 20 (Shimadzu Manufacturing Co., Tokyo, Japan). The bacterial suspension was serially diluted 10-fold with PBS, and the CFUs were determined on Rogosa agar plates after incubation at 37°C for 48 h. *L. monocytogenes* EGD was obtained from K. Takeya, Kyushu University, Fukuoka, Japan. An isolate from the spleens of infected mice was grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 18 h, washed with PBS, suspended in PBS containing 10% glycerol, and stored at −80°C. The isolate was thawed and diluted with PBS for use. The 50% lethal dose was ca. 5×10^3 viable organisms for female 5-week-old ddY mice.

Mouse protection test. Mice were injected intravenously (iv) with 0.2 ml of suspension of *Lactobacillus* spp. at various intervals before iv challenge infection with 0.2 ml of suspension of *L. monocytogenes*, and the survival rate was recorded 7 days after the challenge. As controls, mice were given 0.2 ml of PBS, instead of a suspension of *Lactobacillus* spp.

Assay of *L. monocytogenes*. Mice were injected iv with *L. casei* and infected iv with *L. monocytogenes* after 2 or 13 days. These animals were decapitated at various times after this induced infection. The livers were removed and homogenized in 5 ml of PBS with a glass homogenizer. A 1-ml amount of serial 10-fold dilutions was plated on trypto-soy

TABLE 1. Protective activity of *Lactobacillus* spp. against *L. monocytogenes* infection in mice[a]

Strain	Expt	Dose ($\times 10^7$)	Survivors/total (days)		
			3	5	7
Control	I		3/20	0/20	0/20
	II		0/20	0/20	0/20
L. lactis ATCC 12315	I	3.4	5/19	0/19	0/19
	II	5.8	2/10	0/10	0/10
L. bulgaricus ATCC 11842	I	6.4	3/19	0/19	0/19
	II	5.0	1/10	0/10	0/10
L. acidophilus ATCC 19992	I	11.2	8/20	1/20	0/20
	II	2.8	1/10	0/10	0/10
L. salivarius ATCC 11742	I	2.2	6/20	0/20	0/20
	II	2.2	2/10	0/10	0/10
L. plantarum ATCC 8014	I	10.6	2/20	0/20	0/20
	II	3.8	1/10	0/10	0/10
L. fermentum ATCC 9338	I	2.2	1/20	0/20	0/20
	II	5.6	0/10	0/10	0/10
L. cellobiosus ATCC 11739	I	2.2	6/20	0/20	0/20
	II	7.6	1/10	0/10	0/10
L. brevis ATCC 14869	I	11.0	5/19	0/19	0/19
	II	1.8	1/10	0/10	0/10
L. buchneri ATCC 4005	I	11.8	4/20	0/20	0/20
	II	1.3	2/10	0/10	0/10
L. casei YIT 0003	I	15.0	17/19[b]	8/19[b]	5/19[c]
	II	5.8	17/20[b]	9/20[b]	3/20
Control			0/10	0/10	0/10
L. casei ATCC 393		12.2	8/10[b]	2/10	1/10
L. casei ATCC 7469		11.4	10/10[b]	0/10	0/10
L. casei YIT 0003		10.2	8/10[b]	2/10	1/10
L. casei YIT 0006		8.4	10/10[b]	1/10	1/10
L. casei YIT 0091		11.8	9/10[b]	3/10	0/10
L. casei YIT 0112		7.2	10/10[b]	2/10	0/10
L. casei YIT 9018		7.6	8/10[b]	1/10	0/10

[a] Optical density (at 540 nm) of indicated *Lactobacillus* suspensions was adjusted to 1.0, and 0.2 ml each was injected into mice 2 days before *L. monocytogenes* (first group of experiments, 1.1×10^6; second group of experiments with *L. casei* strains only, 1.0×10^6) infection.

[b] Significantly different from controls at $P < 0.01$ (χ^2 test).

[c] Significantly different from controls at $P < 0.05$ (χ^2 test).

agar (Eiken Chemical Co., Tokyo, Japan) containing 5% NaCl (for growth inhibition of *L. casei*) for assay of *L. monocytogenes*.

CAR treatment. Carrageenan (CAR) (type II; Sigma Chemical Co., St. Louis, Mo.) was dissolved in distilled water at a concentration of 4.5 mg/ml by heating in boiling water. Mice were injected intraperitoneally (ip) with 4.5 mg of CAR 1 day before the *L. monocytogenes* infection (19). As controls, mice were injected ip with 1 ml of distilled water.

X-ray irradiation. Mice were exposed to a 900-rad X-ray with an LMR-4 machine (Toshiba Co., Tokyo, Japan) operating at 4 MV at a distance of 80 cm from the target focus and then challenged after 2 days.

Number and population of peritoneal cells. Mice were injected ip with 10^8 UV-killed *L. monocytogenes* organisms. At various intervals, 5 ml of Eagle minimal essential medium (Nissui Seiyaku Co., Tokyo, Japan) containing 5% fetal bovine serum (M. A. Bioproducts, Walkersville, Md.) and 5 U of heparin per ml were injected into the peritoneal cavity. Peritoneal fluid was harvested after gentle massage of the abdomen, and the exudate cells in the fluid were counted by using a hemacytometer. The fluid was then centrifuged at $150 \times g$ for 10 min, and differential cell counts were made after staining with Giemsa solution.

Labeling and radiometry of liver cells. Labeling of liver cells in DNA synthesis was done by the method of North (22). Briefly, mice were injected iv with 10 μCi of [*methyl*-^{3}H]thymidine ([^{3}H]TdR) 3 Ci/mmol; New England Nuclear, Boston, Mass.), and given 8.4×10^3 *L. monocytogenes* organisms iv after 1 h. At 0, 6, and 48 h after infection, these mice were decapitated, and their livers were removed for radiometry. The liver from other mice obtained at 30 min and 6 and 48 h after injection of 10 μCi of [^{3}H]TdR without *L. monocytogenes* infection served as the control. The liver was homogenized with 5% ice-cold trichloroacetic acid and washed three times with ice-cold trichloroacetic acid, and the [^{3}H]TdR was extracted with 5 ml of trichloroacetic acid (90°C) for 1 h. The extract was diluted with scintillation fluid [1,000 ml of toluene, 500 ml of Triton X-100, 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis(5-phenyl-2-oxazolyl)-benzene], and the ^{3}H activity of the DNA was counted in a Packard Tri-Carb 3255 liquid scintillation counter.

Histopathology. Mice were injected iv with either 1.1×10^7, 1.1×10^8, or 1.1×10^9 *L. casei* cells, and infected with *L. monocytogenes* (10^6) 2 and 13 days after administration of *L. casei*. Livers were removed 48 h after *L. monocytogenes* infection and fixed in 10% Formalin. As controls, mice infected with *L. monocytogenes* (without *L. casei* administration) and injected with *L. casei* (without *L. monocytogenes* infection) were used. Tissue sections were made from all fixed preparations and stained with hematoxylin and eosin.

RESULTS

Protective activity of *Lactobacillus* spp. against *L. monocytogenes* infection. Mice were treated iv with various species of viable *Lactobacillus* 2 days before infection with *L. monocytogenes* (Table 1). A significant extension of survival time occurred in animals pretreated with *L. casei* YIT 0003, compared to those given other *Lactobacillus* spp. Table 1 also shows the protective effects of seven strains of *L. casei* against *L. monocytogenes* infection. All of the strains exhibited similar protective activities. Significant protective effects were also obtained by ip injection, but not by intramuscular or subcutaneous injections (data not shown).

Dose-dependent effects of *L. casei* on the protective activity against *L. monocytogenes* infection. Mice were given either 5.5×10^7, 2.8×10^8, or 1.1×10^9 *L. casei* cells on various days before infection and then were infected with *L. monocytogenes*, and the number of survivors was determined 3 and 7 days after the infection. When mice were given 5.5×10^7 *L. casei* cells (Fig. 1A), the protection against *L. monocytogenes* infection was strongest 2 days after administration of *L. casei*, and it decreased thereafter. When 1.1×10^9 *L. casei* cells were given to mice (Fig. 1C), the increased resistance was seen at ca. 3 to 21 days (but not 2 days) after administration of *L. casei*. In contrast, when mice were given 2.8×10^8 of *L. casei* (Fig. 1B), two peaks of enhanced resistance were seen at ca. 2 and 13 days after *L. casei* administration. These results indicate that the expression of anti-*L. monocytogenes* action of *L. casei* is dependent on the dose of administration.

Inhibition of *L. monocytogenes* multiplication in *L. casei*-treated mice. Figure 2 shows the effects of *L. casei* at three different doses (2 or 13 days before *L. monocytogenes* infection) upon the growth of infected organisms in the liver. When mice were treated with *L. casei* 2 days before infection (Fig. 2A), the growth of *L. monocytogenes* during 6 to

48 h after infection was suppressed by administration of 1.1×10^7 and 1.1×10^8 *L. casei* cells but not by 1.1×10^9 *L. casei* cells. When *L. casei* was given 13 days before infection (Fig. 2B), the growth of *L. monocytogenes* during 6 to 48 h after infection was suppressed in mice treated with 1.1×10^8 and 1.1×10^9 *L. casei* cells but not with 1.1×10^7 *L. casei* cells. It is noted that the killing of *L. monocytogenes* in the liver in the early phase (during the first 6 h) was significantly enhanced in mice given 1.1×10^9 *L. casei* cells. These findings are consistent with those presented in Fig. 1.

Effect of CAR on *L. casei* action. Mice were given CAR 1 or 12 days after the administration of *L. casei* and 24 h later, these mice were infected iv with *L. monocytogenes*. Irrespective of the timing of *L. casei* (−2 or −13 days) injection, a larger number of *L. monocytogenes* was found 10 min after infection in the liver of CAR-nontreated mice than in that of CAR-treated mice (Table 2). CAR treatment caused growth of *L. monocytogenes* 6 h after infection to some extent. The growth of *L. monocytogenes* 48 h after infection in *L. casei*-CAR-treated mice was enhanced in the 2-day groups over both the control and CAR-nontreated groups. However, in the 13-day groups, the 48-h growth of *L. monocytogenes* was enhanced over the CAR-nontreated group but was suppressed with respect to the controls.

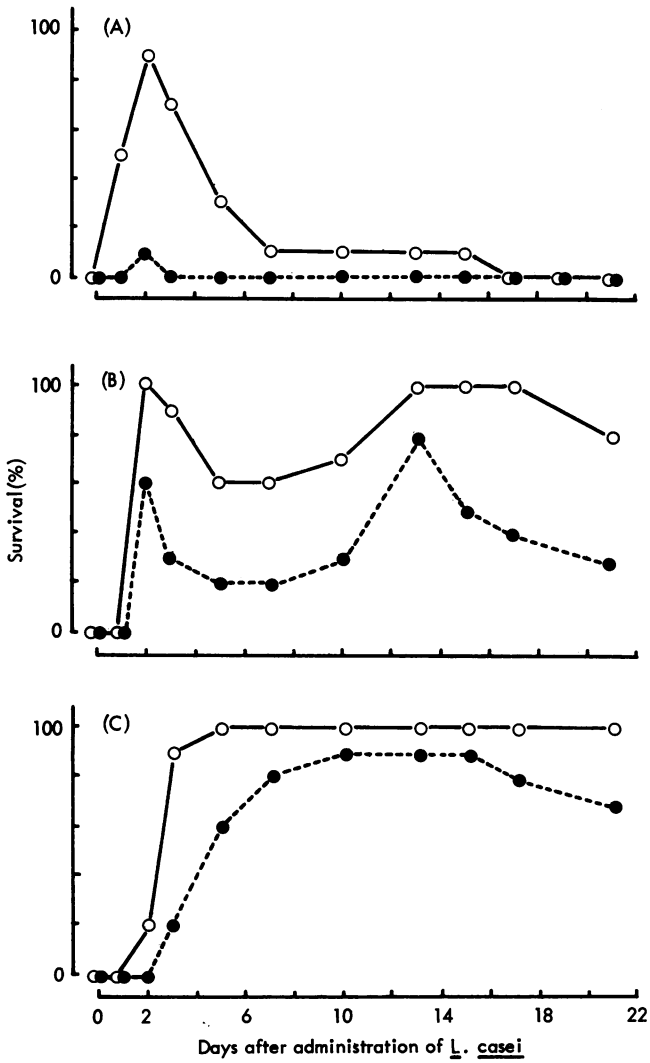

FIG. 1. Appearance and persistency of anti-*L. monocytogenes* action of *L. casei*. Mice were injected iv with (A) 5.5×10^7, (B) 2.8×10^8, and (C) 1.1×10^9 of viable *L. casei* cells on various days before *L. monocytogenes* infection and infected with 10^6 *L. monocytogenes* organisms. Survivors were counted at 3 (○) and 7 (●) days after infection.

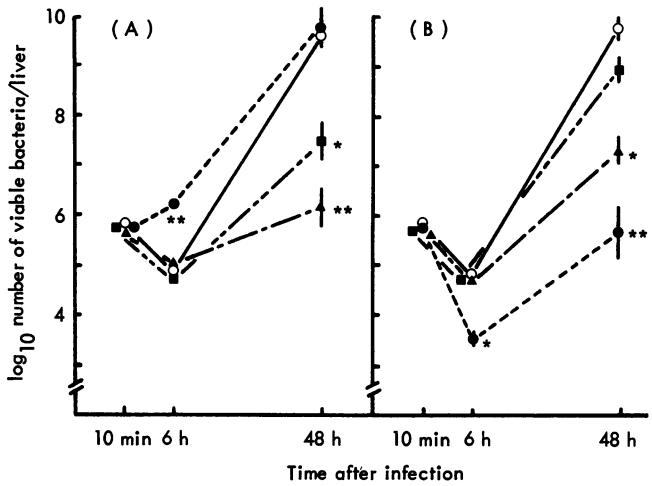

FIG. 2. Dose dependency of anti-*L. monocytogenes* action upon *L. casei*. Mice were given 1.1×10^7 (■), 1.1×10^8 (▲), or 1.1×10^9 (●) of viable *L. casei* cells 2 (A) or 13 (B) days before infection, after which experimental and control (○) mice were infected with 10^6 *L. monocytogenes* organisms. The fate of *L. monocytogenes* trapped in the liver was studied for up to 48 h after infection. Each symbol represents the mean ± the standard error ($n = 3$ to 5). The asterisks indicate significant difference between controls and *L. casei*-treated mice at levels of $P < 0.05$ (*) and $P < 0.01$ (**) (Student's *t* test).

Effect of X-ray irradiation on the *L. casei* action. Mice were given *L. casei* iv 2 or 13 days before *L. monocytogenes* infection with or without X-ray irradiation at 2 days before infection. Although the trapping (10 min) of the infected *L. monocytogenes* by the liver was not affected by *L. casei* administration or X-ray irradiation, these treatments caused a change in the parasite-eliminating efficacy of the host reticuloendothelial system during the first 6 h and in the capacity to reduce the growth of *L. monocytogenes* during 6 to 48 h after infection (Table 3). The killing of *L. monocytogenes* in the liver 6 h after infection was suppressed by *L. casei* administration 2 days before infection but was somewhat enhanced by *L. casei* administration 13 days before infection. X-ray irradiation failed to affect the killing efficacy 6 h after infection of mice with or without *L. casei* treatment. On the contrary, multiplication of *L. monocytogenes* in the liver 48 h after infection was markedly reduced by *L. casei* administration at days −2 and −13. This effect was almost completely abolished by the X-ray irradiation.

Effect of *L. casei* on the accumulation of peritoneal exudate cells induced with *L. monocytogenes*. Mice were given *L. casei* cells iv, and 13 days later, they were injected ip with UV-killed *L. monocytogenes*. After elicitation by *L. monocytogenes*, the number of macrophages in *L. casei*-treated mice began to increase at 6 h, reached a maximum at 3 to 5 days, and decreased to the same level as in *L. casei*-nontreated mice on day 7 (Fig. 3). The number of macrophages in the *L. casei*-nontreated mice was much the same before and after administration of *L. monocytogenes*. The number of polymorphonuclear leukocytes in both *L. casei*-treated and *L. casei*-nontreated mice reached the maximum at 24 h, thereafter decreased rapidly, and returned nearly to

TABLE 2. Antagonistic effect of carrageenan on *L. casei*-mediated enhancement of host resistance[a]

L. casei administration at days before infection	Carrageenan treatment	Log_{10} no. of viable bacteria/liver at time after infection		
		10 min	6 h	48 h
−2				
−	−	3.78 ± 0.15	2.53 ± 0.12	5.33 ± 0.15
−	+	3.01 ± 0.12[b]	3.24 ± 0.26	6.25 ± 0.30[c]
+	−	3.68 ± 0.08	3.10 ± 0.13	3.23 ± 0.18[d]
+	+	3.21 ± 0.20	3.08 ± 0.20	6.10 ± 0.24[c,e]
−13				
−	−	3.50 ± 0.02	2.42 ± 0.08	5.18 ± 0.14
−	+	2.80 ± 0.11[c]	3.29 ± 0.29	6.85 ± 0.42[c]
+	−	3.32 ± 0.13	1.07 ± 0.14[e]	1.94 ± 0.30[e]
+	+	2.90 ± 0.15	2.22 ± 0.10[b]	3.87 ± 0.40[b,e]

[a] Mice were injected iv with 2.5×10^8 viable *L. casei* cells at 1 or 12 days before treatment with carrageenan (4.5 mg). At 24 h after administration of carrageenan, mice were infected iv with 10^4 *L. monocytogenes* organisms. Values are expressed as mean ± standard error ($n = 4$).

[b] Significantly different from carrageenan-nontreated counterpart at $P < 0.05$ (Student's t test).

[c] Significantly different from carrageenan-nontreated counterpart at $P < 0.01$ (Student's t test).

[d] Significantly different from controls at $P < 0.05$ (Student's t test).

[e] Significantly different from controls at $P < 0.01$ (Student's t test).

the normal level on day 3. No differences were found in the number of lymphocytes between *L. casei*-treated and -nontreated mice (data not shown).

Effect of *L. casei* on accumulation of macrophages in the liver after infection. Mice were given *L. casei* iv and 13 days later they were given 10 μCi of [^{3}H]TdR iv. At 1 h after labeling, mice were infected iv with *L. monocytogenes*. [^{3}H]TdR incorporation in the liver DNA before *L. monocytogenes* infection (0 h) was markedly higher in mice given 2.5×10^8 *L. casei* cells than in *L. casei*-nontreated controls (Fig. 4). [^{3}H]TdR incorporation proceeded at almost the same rate in *L. casei*-treated and in nontreated mice during the first 6 h after infection. In contrast, [^{3}H]TdR uptake in *L. casei*-treated mice during 6 to 48 h after infection was much higher than in the nontreated control mice. [^{3}H]TdR incorporation into the liver of *L. monocytogenes*-noninfected mice (without *L. casei* injection) decreased with the lapse of time.

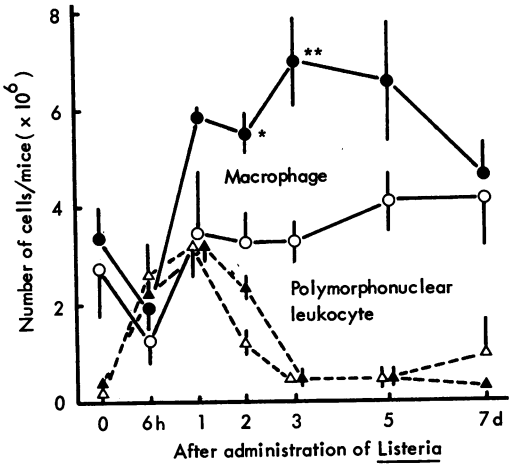

FIG. 3. Effect of *L. casei* on the accumulation of peritoneal cells. Mice were injected iv with (●, ▲) or without (○, △) 6.5×10^8 viable *L. casei* cells and 13 days later ip with 10^8 UV-killed *L. monocytogenes* organisms. At various intervals, the number of peritoneal exudate cells was counted by using a hemacytometer, and differential counts were made with smears stained with Giemsa solution. Each symbol represents the mean ± the standard error ($n = 4$). The asterisks indicate significant difference between controls and *L. casei*-treated mice at levels of $P < 0.05$ (*) and $P < 0.01$ (**) (Student's t test).

TABLE 3. Subversion of *L. casei*-mediated enhancement of host resistance by X-ray irradiation[a]

L. casei administration at days before infection	X-ray irradiation	Log_{10} no. of viable bacteria/liver at time after infection		
		10 min	6 h	48 h
−2				
−	−	3.83 ± 0.10	2.44 ± 0.33	5.91 ± 0.29
−	+	3.76 ± 0.07	2.28 ± 0.28	6.90 ± 0.30[b]
+	−	3.78 ± 0.04	3.12 ± 0.16	4.09 ± 0.24[c]
+	+	3.81 ± 0.06	3.04 ± 0.23	7.24 ± 0.18[d]
−13				
−	−	3.71 ± 0.03	2.55 ± 0.04	5.45 ± 0.28
−	+	3.76 ± 0.08	2.26 ± 0.07	6.60 ± 0.51[b]
+	−	3.80 ± 0.04	1.91 ± 0.27[c]	2.81 ± 0.18[e]
+	+	3.73 ± 0.09	2.03 ± 0.07[c]	5.30 ± 0.47[d]

[a] Mice were injected iv with 2.5×10^8 viable *L. casei* cells 1 h or 11 days before X- ray irradiation. At 2 days after irradiation (900 rad), mice were infected iv with 10^4 of *L. monocytogenes* organisms. Values are expressed as mean ± standard error ($n = 4$).

[b] Significantly different from X-ray-nontreated counterpart at $P < 0.05$ (Student's t test).

[c] Significantly different from controls at $P < 0.05$ (Student's t test).

[d] Significantly different from X-ray-nontreated counterpart at $P < 0.01$ (Student's t test).

[e] Significantly different from controls at $P < 0.01$ (Student's t test).

Histopathology of the liver. In the liver of mice 2 days after administration of *L. casei* (1.1×10^9), a large number of small to intermediate nodular infiltrations consisting of inflammatory cells, mainly monocytes together with a few neutrophils, were observed in the sinusoids, with irregular distribution to the lobules. Similar, but milder hepatic lesions were observed in mice given 1.1×10^7 and 1.1×10^8 *L. casei* cells. On the other hand, in the liver of mice 13 days after administration of *L. casei* (1.1×10^9), a marked feature was that nearly all of the monocytes composed of intermediate to large nodules changed to epithelioid cells, and a few typical monocytes were seen only inside or surrounding the nodules. Epithelioid cells were also observed in nearly half of the small nodules. Therefore, most of the nodular lesions caused by *L. casei* exhibited a picture of granuloma (Fig. 5). Histopathological changes were caused by 1.1×10^9 *L. casei* cells, and no change was found in the case of 1.1×10^7 *L. casei* cells. Figure 6 shows histopathological findings in the liver 48 h after *L. monocytogenes*-induced infection. The major lesions were composed of many bacterial clumps and necrosis of the hepatic cells, that is, severe necrotizing of inflammatory lesions of the septic type. When mice were infected with *L. monocytogenes* 2 days after administration of *L. casei*, there was a decrease in bacterial clumps and necrosis of the hepatic cells and an increase in the number of monocytes, irrespective of the dose of *L. casei* (1.1×10^7,

1.1×10^8, or 1.1×10^9). Histopathological changes in the liver of mice infected with *L. monocytogenes* 13 days after administration of 1.1×10^9 *L. casei* cells were similar to those in the liver of mice given 1.1×10^9 *L. casei* cells (see Fig. 5), and there was no evidence of bacterial clumps (Fig. 7). The lesions in the liver of mice infected with *L. monocytogenes* 13 days after administration of 1.1×10^7 *L. casei* cells were similar to those in the liver of mice infected with *L. monocytogenes* (see Fig. 6), however, the lesions in the case of 1.1×10^8 *L. casei* cells were milder than those in the case of 1.1×10^7 *L. casei* cells.

DISCUSSION

The host resistance against *L. monocytogenes* infection primarily depends on macrophages activated by sensitized lymphocytes (12, 14). The resistance in the early period of infection (during the first 48 h after infection) is also attributed to nonimmune macrophages (19). Newborg and North (21) and Mitsuyama et al. (19) reported that there are two differential phases of macrophages contributing to the host resistance to *L. monocytogenes* in the early period of infection. In the first phase (during the first 12 h after infection), the infected organism is killed mainly by radioresistant and CAR-sensitive macrophages (19, 28) which are fixed macrophages in the liver. In the second phase (during 12 to 72 h after infection), the radio- and CAR-sensitive macrophages, which newly accumulate at the infection sites (liver), play a major role in preventing further bacterial multiplication.

With respect to the enhancement of resistance to *L. monocytogenes* infection that was seen at ca. 2 days after *L. casei* administration, the following considerations were made. The administration of *L. casei* (1.1×10^7 or 1.1×10^8) cells failed to enhance the killing of *L. monocytogenes* in the liver 6 h after infection but did suppress growth of organisms 48 h after the infection (Fig. 2B). Histopathological studies revealed that the number of macrophages in the liver increased 2 days after *L. casei* administration. The killing of *L. monocytogenes* 6 h after infection in *L. casei*-treated mice was not affected by X-ray irradiation or CAR treatment, but the inhibitory effect of *L. casei* on *L. monocytogenes* multiplication 48 h after infection was curtailed. The enhanced resistance against *Klebsiella pneumoniae* in the early phase (ca. 2 days) induced by *Propionibacterium acnes* is impaired by CAR, which abrogates the phagocytic cell functions (28), but it is not impaired by cyclophosphamide and anti-mouse thymocyte serum (13), which abrogate lymphocytes (20, 29). These findings indicate that the enhanced resistance at this phase is mediated by nonspecifically stimulated macrophages newly emigrating from the blood stream to the liver (19, 22) before and after *L. monocytogenes* infection but not by fixed macrophages in the liver. Stimulation of the mononuclear phagocytic system by *Corynebacterium parvum* is inhibited by prior depletion of complements, indicating the role of complements and their activated subfragments in this phenomenon (8). It is known that *Lactobacillus* spp. activate complement by an alternative pathway (10), and it was thought that the complement activation by *L. casei* plays some role in the enhancement of the host anti-*L. monocytogenes* resistance 2 days after *L. casei* administration. However, this may be excluded because mice given the highest dose (1.1×10^9) of *L. casei* 2 days before infection did not develop the enhanced resistance to *L. monocytogenes* (Fig. 1C).

Concerning the enhanced resistance to *L. monocytogenes* seen around 13 days after *L. casei* administration, the following can be noted. Accumulation of macrophages in the peritoneal cavity by *L. monocytogenes* (UV-killed) elicitation was enhanced when mice were given *L. casei* 13 days before. The administration of *L. casei* (1.1×10^9) 13 days before infection enhanced the killing of *L. monocytogenes* in

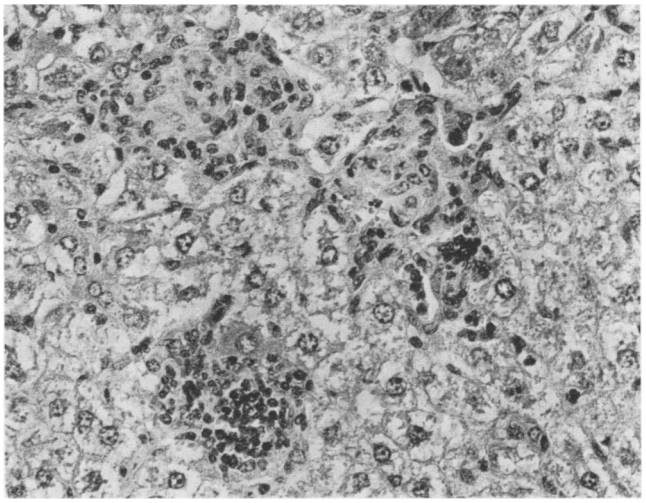

FIG. 5. Histopathological findings of the liver of mice 13 days after administration of *L. casei* (1.1×10^9). A large number of nodular infiltrative lesions consisting mainly of monocytes together with a few neutrophils are observed in the sinusoids, with irregular distribution to the lobules. Many epithelioid cells and a few typical monocytes are seen only inside or surrounding the nodules (hematoxylin and eosin stain; original magnification, ×200).

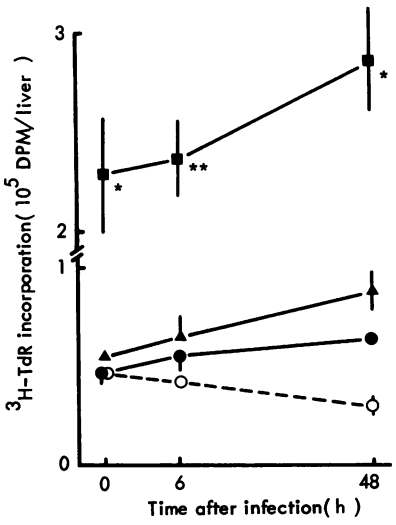

FIG. 4. Accumulation of macrophages labeled during infection in the liver. Mice were injected iv with 1.0×10^7 (▲) and 2.5×10^8 (■) or without (●) viable *L. casei* cells. After 13 days mice were given 10 μCi of [^{3}H]TdR iv. At 1 h after labeling, mice were infected iv with 8.4×10^3 *L. monocytogenes* organisms, and the liver of mice was used for radiometry 0, 6, and 48 h after infection. As controls (○), the liver of mice 30 min and 6 and 48 h after injection of 10 μCi of [^{3}H]TdR without *L. monocytogenes* infection was used. Each symbol represents the mean ± the standard error ($n = 3$). The asterisks indicate significant difference between controls and *L. casei*-treated mice at levels of $P < 0.05$ (*) and $P < 0.01$ (**) (Student's t test).

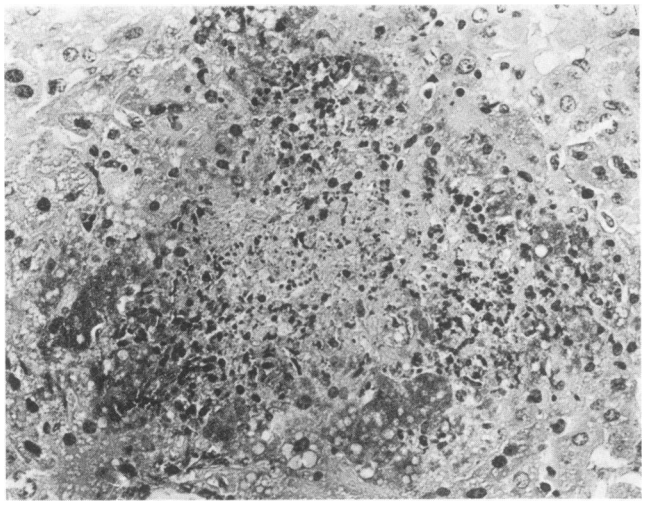

FIG. 6. Liver sample of mice 48 h after *L. monocytogenes* infection. Note the numerous bacterial clumps and necrosis of the hepatic cells (hematoxylin and eosin stain; original magnification, ×200).

the liver 6 h after infection, and the growth of *L. monocytogenes* was suppressed by *L. casei* administration (1.1×10^8 or 1.1×10^9) 48 h after infection. The inhibitory effect of *L. casei* on the multiplication of *L. monocytogenes* 48 h after infection was inhibited by X-ray irradiation or by CAR treatment. In contrast, the killing of *L. monocytogenes* 6 h after infection in *L. casei*-treated mice was not affected by X-ray irradiation but was suppressed by CAR treatment. Therefore, the increased host anti-*L. monocytogenes* resistance at ca. 13 days after administration of *L. casei* seems to be mediated not only by the fixed macrophages but also by newly emigrating macrophages from the blood monocyte pool (19, 22) in response to *L. monocytogenes* infection. North (22) reported that a single pulse of [^{3}H]TdR given just before infection resulted in an immediate labeling of cells already present in the liver, and the increased [^{3}H]TdR uptake in the liver with *L. monocytogenes* infection represents the rate of accumulation of migratory macrophages from the circulating monocyte pool to the infection site. In the present study, [^{3}H]TdR incorporations in the liver before and after infection increased by *L. casei* (2.5×10^8) administration. Moreover, histopathological studies revealed the increased monocyte infiltration into the liver by administration of *L. casei* 13 days before infection. Thus, the number of macrophages in the liver at the time of infection was enhanced by *L. casei* administration before infection, and *L. casei* treatment further accelerated macrophage accumulations after infection. On the other hand, it was noted that epithelioid granulomas were formed in the liver 13 days after *L. casei* administration. In general, it is thought that this formation is due to immunological reactions, and in some cases, DTH reactions are required for their formation (1, 16). Mitsuyama et al. (18) found that macrophages accumulating at the site of a DTH reaction are important in anti-*L. monocytogenes* host resistance. This finding strongly supports the view that, in *L. casei*-treated mice accumulation and subsequent activation of liver macrophages are enhanced by lymphokines produced by *L. casei*-primed T cells. Indeed, I found that the peritoneal macrophages harvested 13 days after ip *L. casei* (2.5×10^8 or 1×10^9) administration acquired a markedly enhanced listericidal activity, O_2^-- and H_2O_2-producing ability responding to phorbol myristate acetate phagocytic capacity against latex particles, and acid phosphatase activity compared to the resident macrophages and also to thioglycolate-induced macrophages (unpublished data). Therefore, it is thought that the DTH reactions against *L. casei* antigen(s) were developed in the liver or spleen of mice receiving high iv doses of viable *L. casei*. Thus, a sufficient amount of activated fixed macrophages would be provided at the time of infection. The enhanced resistance to *L. monocytogenes* 13 days after *L. casei* administration may be attributed to macrophages already accumulated at the site of the DTH reaction, to fixed macrophages activated by lymphokines, and to macrophages accumulated by *L. monocytogenes* infection.

The lack of enhanced resistance to *L. monocytogenes* infection 13 days after administration of 5.5×10^7 *L. casei* cells can be explained as follows. There was no evidence of viable *L. casei* in the liver 13 days after *L. casei* administration (data not shown); histopathologically, there was no evidence of infiltration of macrophages into the liver 13 days after *L. casei* (1.1×10^7) administration, and enhancement of the killing (6 h) and growth inhibition (48 h) of *L. monocytogenes* in the liver 13 days after *L. casei* administration were slight (data not shown). Therefore, macrophages related to the DTH reaction elicited by *L. casei* antigens do not seem to accumulate before infection. The lack of significantly enhanced resistance 2 days after the administration of 1.1×10^9 *L. casei* cells is explained as follows. When 1.1×10^9 *L. casei* cells were administered 2 days before infection (Fig. 2A), there was a marked increase in the number of infecting *L. monocytogenes* organisms. Consequently, it seems that macrophages which phagocytozed numerous *L. casei* cells were unable to kill infecting *L. monocytogenes* organisms, presumably due to the depression of their cell functions by overphagocytosis known as the "carbon blockade" (32).

In any case, it is thought that the major action of *L. casei* 2 and 13 days after its administration is mediated by radiosensitive macrophages. The administration of *L. casei* induced the formation of epithelioid granulomas after 13 days. Therefore, the manifestation of anti-*L. monocytogenes*

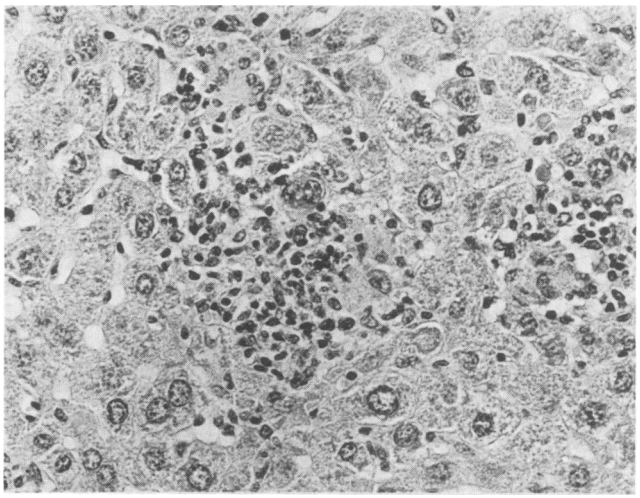

FIG. 7. Liver tissue from a mouse infected with *L. monocytogenes* 13 days after *L. casei* (1.1×10^9) administration. Note that changes are similar to those in Fig. 5 (hematoxylin and eosin stain; original magnification, ×200).

resistance 13 days after *L. casei* administration is mainly based on macrophages accumulating at the site of the DTH reaction elicited by *L. casei* antigen before *L. monocytogenes* infection. Interestingly, the number of macrophages accumulated by *L. casei* before infection is further accelerated after *L. monocytogenes* infection. Factor(s) which accelerate the accumulation of macrophages after *L. monocytogenes* infection in mice treated with *L. casei* remain to be determined.

It is noted that only *L. casei*, among various lactobacilli, showed a remarkable protective activity against *L. monocytogenes* infection (Table 1). Although the administration dose of *Lactobacillus* spp. on the basis of CFUs varied among the test strains, gross comparison of the effectiveness of *Lactobacillus* spp. strains to enhance the host resistance may be possible. In the present experiment the doses administered were set to be almost equal to each other by adjusting the optical density of the *Lactobacillus* spp. suspensions and by injecting them in an equal volume into mice. The present findings indicate that only *L. casei* possesses the particular cellular component(s) required to enhance the host resistance.

ACKNOWLEDGMENTS

I thank H. Saito for pertinent suggestions and advice during this study, H. Tomioka for valuable discussions, H. Umisa for suggestions on histopathology, and M. Ohara for critical comment on the manuscript.

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